National Reference System for Cholesterol

Cholesterol Reference Method Laboratory Network

HDL Cholesterol
Certification Protocol for Manufacturers

November 2002

CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK

HDL Cholesterol Certification Protocol for Manufacturers

General Information

The second report of the National Cholesterol Education Program's (NCEP's)* Adult Treatment Panel (ATP II) reiterated the recommendations of the first report that total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) be used as the primary indicators of coronary heart disease (1,2). In addition, the second report increased emphasis on high-density lipoprotein cholesterol (HDLC) concentration in the detection, evaluation, and treatment of people who may be at increased risk for coronary heart disease. The third report from the ATP established specific medical decision points, 40 mg/dL (1.0 mmol/L) and 60 mg/dL (1.6 mmol/L), which were derived from national population studies in which the HDLC assays were standardized to the reference method (RM) at the Centers for Disease Control and Prevention (CDC) (3). The NCEP's Working Group on Lipoprotein Measurement developed recommendations about clinical laboratory measurements of HDLC (4). This group recommended that the link be maintained between laboratory measurements and the existing epidemiologic and clinical database. Therefore, the Cholesterol Reference Method Laboratory Network (CRMLN) links to the database through a protocol for certification of HDLC methods, based on a fresh sample comparison with a CRMLN laboratory. The CRMLN laboratories use either the CDC RM or a designated comparison method (DCM), both of which are standardized to the CDC (see the attached table for the methods used in each CRMLN laboratory). The CRMLN believes that standardization can be achieved most effectively through the manufacturers of analytical instruments and reagents. This document provides the details of how this program works.

The NCEP Working Group recommended that laboratories perform HDLC analyses with bias ≤5.0% from the true value (reference method) and precision, as measured by coefficient of variation (CV) at concentrations ≥ 42 mg/dL (1.09 mmol/L), ≤4.0%; and as measured by standard deviation (SD) at concentrations <42 mg/dL (1.09 mmol/L), ≤ 1.7 mg/dL (0.04 mmol/L) (4). These goals for accuracy and precision suggest that for a single measurement, the allowable total error would be 13%. Precision can be improved by adherence to accepted principles of good laboratory practice and quality assurance. Accuracy can be improved by evaluation of traceability to the accuracy base through a fresh sample comparison with one of the CRMLN laboratories. ***Although the CRMLN believes that evaluation of total error is important, it encourages manufacturers to strive to meet the NCEP's accuracy and precision recommendations separately.***

Two major difficulties exist in standardizing the traditional HDLC methods. The first is the biases between the various precipitating reagents that have traditionally been used to separate the LDLC and very low-density lipoprotein cholesterol (VLDLC) from the HDLC. The second is the altered matrix characteristics of calibrators and controls, a result of the

* Acronyms & Abbreviations: NCEP – National Cholesterol Education Program, ATP– Adult Treatment Panel, TC – total cholesterol, LDLC – low-density lipoprotein cholesterol, HDLC – high-density lipoprotein cholesterol, RM – reference method, CDC – Centers for Disease Control and Prevention, CRMLN – Cholesterol Reference Method Laboratory Network, DCM – designated comparison method, CV – coefficient of variation, SD – standard deviation, VLDLC – very low density lipoprotein cholesterol, QC – quality control.

manufacturing process, which cause them to react differently from fresh patient specimens in some analytical systems. Calibrating a matrix-sensitive assay system with reference target values of commercially prepared materials results in significant biases when fresh patient specimens are analyzed. Calibrators must be assigned set points that result in accurate results on fresh patient samples.

Two potential difficulties exist in standardizing homogeneous HDLC methods. The first is the biases associated with different principles used by the various homogeneous reagents that are used to differentiate the absorbance from the LDLC and VLDLC from the absorbance used to quantify HDLC and the resulting different correlation relationships to the RM. The second potential difficulty is the same as with the traditional methods--the altered matrix characteristics of calibrators and controls. Calibrators must be assigned set points that result in accurate results on fresh patient samples.

The only universally reliable approach to establishing appropriate calibration for acceptable accuracy must be based on comparison studies conducted with fresh specimens. This is the only approach that addresses all of the difficulties in standardization of both the traditional precipitation methods and the homogeneous methods.

The RM for HDLC used at CDC is a three-step procedure involving ultracentrifugation, precipitation with heparin-manganese reagent, and quantification with the Abell-Kendall reference method for cholesterol. It was developed for the purpose of assigning reference values to reference materials that are used in CDC's standardization programs. The method is expensive to operate (it requires the use of an ultracentrifuge), requires a large volume of sample (5 mL of specimen), and has low throughput (18-hour ultracentrifugation). The CRMLN developed a DCM for HDLC that overcomes some of the practical disadvantages of the RM by requiring lower sample volume and having a higher throughput (5). The DCM used by the CRMLN involves precipitation with dextran sulfate-magnesium reagent and quantification with the Abell-Kendall reference method for cholesterol. Both methods are used in the CRMLN, and both are standardized to the same high level of performance. (DCMs are defined by NCCLS's National Reference System for the Clinical Laboratory [NRSCL]) in the Guideline NRSCL13-P, *The Reference System for the Clinical Laboratory: Criteria for Development and Credentialing of Methods and Materials for Harmonization of Results* (6).

The CRMLN provides services for manufacturers who wish to certify their HDLC methods. The certification protocol for manufacturers is based upon the NCCLS Guideline EP9-A, *Method Comparison and Bias Estimation Using Patient Samples* (7). The protocol includes a comparison with the CRMLN laboratory using at least 40 fresh serum specimens. The protocol also includes analysis of a quality control (QC) material in 20 runs. Demonstration of absolute average bias ≤5% of the reference method and total CV ≤4% qualifies an analytical system for certification. The set points assigned by the manufacturer to that system's calibrators should be appropriate to ensure accurate analytical results on patient specimens in the hands of users.

Because the NCEP recommends that clinical laboratories achieve total error ≤13% on patient specimens, total error will be calculated. However, certification will be based on meeting the recommended goals for accuracy and precision.

Manufacturers must assume all responsibility for the results and should make the initial contact with the CRMLN laboratory. Manufacturers are advised to contact a CRMLN laboratory before beginning this protocol. A list of CRMLN laboratories can be obtained from the CRMLN Web site at http://www.cdc.gov/nceh/dls/crmln/memberlabs.htm. One set of fresh samples may be split and used to certify several applications, thus necessitating only one set of analyses by the CRMLN laboratory.

The Initial Certification Process

Anyone collecting and handling any biological material of human origin ***MUST*** observe Universal Precautions (8).

Purpose

The HDLC certification program is intended to assess the bias of the comparison method against the DCM or RM under defined conditions. Not all samples that may be encountered in clinical laboratories are included in the scope of this program. Thus, the protocol is designed to verify the calibration, not the robustness, of the methods evaluated.

Manufacturer's Preliminary Responsibilities

Before pursuing certification through the CRMLN, manufacturers should establish that their analytical instrument systems meet the following standard specifications.

- Instrument system(s) must be capable of producing discrete number values.
- Instrument system(s) must have had all required preventive maintenance procedures and must be in peak operating condition.
- Precision testing (such as that outlined in NCCLS Guideline EP5-T2, *User Evaluation of Precision Performance of Clinical Chemistry Devices*) should be done to ensure that total precision is ≤4% (9).

Note: *Manufacturers who have the DCM or the RM set up in house may establish its traceability to the accuracy base by evaluation through the CRMLN. Although this internal verification does not substitute for certification of the manufacturer's products, manufacturers can use the DCM or RM in house to check their products before evaluation of traceability by a CRMLN laboratory.*

Manufacturer's Specimen Collection

Specimens should be fresh; fasting donors are preferred. Use of frozen specimens is not recommended unless the manufacturer has conducted a careful freeze/thaw study and determined that use of frozen specimens in the test system would not compromise the results of the comparison with the accuracy base. The certification protocol is designed to evaluate analytical method bias only. Therefore, variation from preanalytical sources must be eliminated or minimized. Manufacturers should carefully follow the protocol for sample collection and processing described in this section.

The recommended sample matrix is serum; however, the comparison should be performed using the sample matrix for which the analytical system is designed. Venous serum is the matrix to be used for all comparisons designed to establish traceability to the accuracy base. Values for all other blood matrices must be traced to venous serum values through paired sample comparisons. Alternate blood matrices would include all capillary samples (including serum) and all anticoagulated samples from both venous and capillary sites. For example, if a manufacturer's system is designed to analyze capillary plasma and the manufacturer wishes to be certified for this matrix, the manufacturer should collect paired venous serum and capillary plasma samples from the patients used in the comparison. The manufacturer should then analyze the capillary plasma samples and submit the venous serum samples to the CRMLN laboratory for analysis.

Collect and analyze 40 or more fresh specimens from patients. The HDLC concentration of these specimens should be distributed over the clinically meaningful range. To accomplish this, at least five samples are required in each of the concentration regions listed below. The remaining number of samples (a minimum of 15) should be spread out over the entire range.

Range	
20-29 mg/dL	(0.52-0.77 mmol/L)
30-39 mg/dL	(0.78-1.03 mmol/L)
40-49 mg/dL	(1.04-1.29 mmol/L)
50-59 mg/dL	(1.30-1.55 mmol/L)
60-69 mg/dL	(1.56-1.81 mmol/L)

A worksheet for specimen distribution is attached as an aid.

The HDLC DCM requires that the TG concentration be less than 200 mg/dL (2.26 mmol/L). The TG concentration of samples to be compared with the HDLC RM is not restricted.

These specimens must:

- be free of interfering substances known to affect the system being tested (e.g., hemolysis, icterus, lipemia);
- be collected in sufficient quantity (see sample volume requirements for DCM and RM below);
- not include samples that the product's package insert indicates should be excluded;

- be collected in sufficient quantity (see sample volume requirements for DCM or RM testing below); and

- be collected using good laboratory practice (such as outlined in NCCLS Guideline H3-A4, *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture* (10)).

To make the certification process reflect reality, the protocol followed should mimic, as much as possible, the conditions in the clinical laboratory. Manufacturers are encouraged to develop package inserts that include information about time frames for storage and analysis of patient samples. Storage times and methods should be evaluated as part of the method development.

Balancing the need for reflecting conditions in clinical laboratories is the need to have the samples analyzed by the manufacturer be as similar as possible to those analyzed by the CRMLN laboratory. Serum should be separated from red cells within 2 hours of collection. The serum for the comparison may be stored up to 8 hours at 4°C after separation. Within this 8-hour period, an aliquot (2.5 mL for DCM or 5.5 mL for RM) of each serum sample must be frozen (at –70 °C or lower) for analysis by the CRMLN laboratory. At the same time the sample for CRMLN laboratory analysis is frozen, the test method(s) analysis should begin.

For methods involving separation by precipitation: if the supernates resulting from the precipitation cannot be analyzed the same day as collected, they can be stored at 4 °C or frozen before analysis, if the manufacturer has documented that no adverse effects result of such storage conditions.

The minimum amount of serum needed per sample for DCM analysis is 2.5 mL. The minimum amount of serum needed per sample for RM analysis is 5.5 mL. Samples intended for analysis by the CRMLN laboratory should be dispensed into cryogenic vials, frozen immediately (at -70 °C or lower), and stored frozen until all runs are complete. Specimens must be clearly identified using coded identification numbers and **no patient identifiers.** Specimens must be securely contained in cryogenic vials to prevent breakage, leakage, and evaporation.

More than one tube of blood from a single individual may be necessary to obtain enough volume for the CRMLN laboratory analyses as well as the test method analyses. If this is the case, harvest the serum, combine the contents, and mix well before aliquoting the samples. This will not apply when an alternative matrix is evaluated by the test method. Serum must be provided to the CRMLN laboratory; a separate sample tube from the same person must be collected at the same time if the test method uses either plasma or whole blood.

Difficulty in obtaining a sufficient amount of sample from one individual can be overcome by combining two specimens--**but no more than two**--from separate individuals. This combined specimen pool should be treated as an unpooled (single) sample. The combined specimen pool should be mixed well before aliquoting.

Some of the same samples used for certification of other analytes by the CRMLN may be used. Because all samples are unlikely to meet the sample distribution guidelines for all analytes, additional samples should be collected. This will ensure that the guidelines are met for all analytes.

We strongly recommend *more* than 40 samples be sent to the CRMLN laboratory to avoid delays resulting from insufficient samples, outliers, or lab accident.

The CRMLN strongly recommends that manufacturers set aside and store *(at -70 °C or lower)* additional aliquots of each sample (or supernate if test method uses precipitation), prepared at the same time that the initial samples or supernates for the certification are prepared. They can be used for reanalysis if changes in calibration are required to meet NCEP criteria. When new lots of calibrators, materials, or reagents are prepared, these frozen samples can provide an important link to the accuracy base during overlap analyses if a frozen versus fresh comparison has been performed.

Collection of patient samples is the responsibility of the manufacturer. However, CRMLN laboratories may assist in this process by collecting some or all of the specimens, as long as the specimens analyzed by the test method are fresh. Alternatively, manufacturers can work with a local clinic or hospital to collect samples.

Manufacturer's Quality Control

To evaluate total error, estimates of both inaccuracy and imprecision are needed. The inaccuracy can be obtained from the split sample comparison with the CRMLN laboratory. However, to estimate imprecision, the CRMLN requires that manufacturers provide QC data obtained from 20 separate runs. The recommended concentration range for the QC material is 30 to 60 mg/dL (0.78 to 1.55 mmol/L). A frozen human serum pool is more representative of fresh patient samples and is preferable over the use of a processed material (e.g., lyophilized). The latter may result in higher imprecision leading to a lower error budget for bias. These materials should be analyzed in single using the complete analytical system being evaluated. The runs must include those used in the split sample comparison described below. Use the attached Quality Control Results Form for reporting the results.

Manufacturer's Specimen Analysis

The CRMLN recommends that manufacturers perform the comparison analyses at the manufacturing site; however, manufacturers may have a clinical laboratory using their system perform the split comparison with the reference method. Clinical laboratories that perform successful comparisons on behalf of a manufacturer will also receive a Certificate of Traceability for HDL Cholesterol.

Calibration is the key to achieving accuracy; therefore, these comparison runs should represent the conditions recommended to customers. Calibrators should be analyzed along with the patient samples.

More than one system may be certified using one set of samples. **If more than one lot of reagents or calibrators are to be certified, all of the fresh samples should be analyzed with each lot.** Each combination of instrument, reagents, and calibrator that meets the NCEP performance recommendations will be issued a Certificate of Traceability for HDL Cholesterol.

- Follow the protocol for collecting, dispensing, and storing samples as described on pages 4 - 6 of this document. Store the samples at 4 ºC before analysis and begin analysis of all specimens within 8 hours of collection. Perform the precipitation on duplicate aliquots of each sample. Randomize the concentrations in the run sequence. Assign the first set of aliquots from each specimen sequential positions in the run. Run the duplicate measurement of each specimen in reverse order.

- Include the QC material selected above in every analytical run.

- Analyze the specimens over at least 5 runs, one run per day. Two runs per day are acceptable, provided that the runs are separated by 2 hours (NCCLS definition of a run, per EP5-A).

- Run days need not be consecutive, but all testing should be completed within a reasonable time period (less than 1 month).

- Perform testing following the instructions provided in the package labeling (i.e., do not follow in-house modifications).

- If an instrument problem develops during a run or internal QC is unacceptable, retest specimens from that run after the problem has been identified and corrected if storage of samples or supernates will not affect the result. Samples for analysis by the CRMLN laboratory should have been frozen at the time the sample was first precipitated.

- Complete the Information Form, the Fresh Sample Comparison Result Form, and the Quality Control Results Form. Photocopy the blank forms and save them for future comparisons.

Shipment of Specimens to the CRMLN laboratory

When the fresh specimens have been analyzed by the test method(s), ship the recorded results to the CRMLN laboratory along with the frozen serum aliquots.

Contact the laboratory first to arrange for the shipment. Ship the frozen samples on dry ice by overnight express delivery to the CRMLN laboratory. Samples should be shipped between Monday and Thursday to ensure delivery during the workweek. Include results of the analysis of the fresh specimens by the test method and the target values for calibrators used. Before shipping, ensure that the test specimens are clearly and indelibly labeled and that the labels will remain secure during shipment and subsequent storage. . Include the

Information Form, the Fresh Sample Comparison Result Form, and the Quality Control Results Form with the samples.

A Protocol Checklist is provided. Please refer to the checklist to make sure that all requirements in this protocol have been met.

Follow current federal and state regulations for handling, packaging, and shipping potentially biohazardous materials with regard to containment, labeling, and other procedures.

CRMLN Laboratory Analysis, Data Reduction, and Cost

The CRMLN laboratory will analyze each specimen in duplicate; a minimum of 3 runs will be used. Manufacturers will be provided with the results and statistical analyses. The approximate turnaround time for CRMLN laboratory analysis and the data analysis is 3-4 weeks from receipt of samples, but could take longer depending on the analytical workload at the CRMLN laboratory. If a manufacturer wishes to evaluate more than one analyte (e.g., total cholesterol, HDLC and LDLC), the CRMLN laboratory analyses could take longer than 3-4 weeks.

Because both the DCM and RM are manual and tedious and must be maintained within very stringent limits for accuracy and precision, the assay is costly. All of the CRMLN laboratories offer HDLC analyses for the same cost. Refer to the attached fee schedule for current pricing. Manufacturers can test the same set of specimens on several instrument, calibrator, or reagent systems. There will be a nominal fee for certifying each additional system to cover data analysis costs.

After meeting all the certification criteria, the manufacturer will be issued a dated Certificate of Traceability for HDL Cholesterol, stating that the analytical system (including instrument model, reagent lot, and calibrator lot), has successfully demonstrated traceability to the accuracy base for HDLC under the conditions tested. The Certificate will list the bias versus the RM, the total CV, and the calculated total error. A separate certificate will be issued for each analytical system that successfully meets the NCEP performance recommendations. The date used on the certificate as the "Date of Comparison" is the date that the data is analyzed at the CRMLN laboratory. Certificates expire 2 years after this date. Manufacturers are encouraged to maintain current certification.

Once the analytical system has been certified, conventional in-house QC procedures should be adequate to monitor the system. However, if shifts are observed, another direct comparison with the CRMLN should be undertaken to reset the system for optimal accuracy. Changes in lots of calibrators or reagents should be carefully checked to maintain accuracy. If the reagent or calibrator formulations, or the instrumentation are substantially modified, a new direct comparison will be needed to verify accuracy under the new conditions. This Method Certification Protocol should be followed in this case.

The CRMLN publishes a list of manufacturers' systems that have been certified through this protocol. The list is available at http://www.cdc.gov/nceh/dls/crmln/hdltable.htm and includes all systems with a current Certificate of Traceability for HDL Cholesterol.

Proficiency surveys, such as those offered by the College of American Pathologists, are essential in providing feedback on the performance of laboratory analytical systems. Differences in values have been observed with certain systems using lyophilized survey pools. Manufacturers would best serve their clients by assessing the validity of these lyophilized survey pools on their systems and advising users of any offset or bias. Eventually any interim confusion should be reduced, and better set points, survey pools, and control materials will allow for better reliability in maintaining accuracy.

Questions about this protocol should be directed to a CRMLN laboratory (see http://www.cdc.gov/nceh/dls/crmln/memberlabs.htm for a list of CRMLN laboratories). Copies of this protocol are also available at the CMRLN website.

Statistical Criteria used for Certification

The criteria used for grading are listed in the following table.

Parameter	Criterion	Statistical approach
r^2	> 0.975	Linear regression
Bias at 35 mg/dL	≤ 5%	Linear regression equation; NCEP accuracy guideline
Bias at 60 mg/dL	≤ 5%	Linear regression equation; NCEP accuracy guideline
Average % Bias	≤ 5%	Mathematical mean of biases; NCEP accuracy guideline
Average Absolute % Bias	≤ 5%	Mathematical mean of absolute biases; NCEP accuracy guideline
Among-run CV	≤ 4%	CV of QC results; NCEP precision guideline
t-test of bias	Not significant at α = 5%	See below
Within-method outliers	1 allowed	EP9-A (7), see below
Between-method outliers	None allowed, but may eliminate one sample	EP9-A (7), see below

CRMLN members participate in surveillance to evaluate their performance versus the CDC accuracy base. They are required to meet very strict performance criteria, which are listed in the following table.

Performance Criteria for CRMLN Laboratories		
	Accuracy Criterion	Imprecision Criterion
HDL Cholesterol	bias ≤ 1 mg/dL	SD ≤ 1 mg/dL

The bias of the CRMLN laboratory is the most important factor that needs to be controlled to make appropriate decisions about the performance of test systems (11). Although the CRMLN has achieved a very low bias compared with CDC, some bias still exists. Statistical analysis has been used to study the distribution of the biases obtained by the CRMLN laboratories.

For HDL cholesterol, results of statistical analysis of the survey data collected from September 1998 through February 2000 show a non-Gaussian distribution. The total number of events was 200. The mean bias was -0.2 mg/dL with a SD of the bias of 0.6. The biases, by percentile, were -0.6 mg/dL for the 25th, -0.2 mg/dL for the 50th (median), and 0.1 mg/dL for the 75th. However, since test systems are evaluated based on whether or not they meet an accuracy criterion of ≤ 5%, statistical analysis of the percent bias was also performed. The mean percent bias was -0.5% with a SD of the bias of 1.4%. The percent biases, by percentile, were -1.4% for the 25th, -0.4 % for the 50th (median), and 0.3% for the 75th. We have determined that the CRMLN can allow test systems an additional -0.5% to 1.5% bias.

This is applied asymmetrically so that the allowable bias is -5.5% to 6.5%.

The following table lists the NCEP performance recommendations, as well as the performance allowances for manufacturers and clinical laboratories, as described above.

Performance Criteria for Manufacturers and Clinical Laboratories			
	NCEP Inaccuracy	NCEP Imprecision	CRMLN Inaccuracy Allowance
HDL Cholesterol	bias ≤ 5 %	CV ≤ 4 %	-5.5% to 6.5%

The CRMLN has also implemented the use of a t-test to evaluate whether or not the test system's bias is significantly different from the NCEP goal. The variance component of the t-test utilized in the CRMLN programs is the NCEP's maximum allowable imprecision. The rationale for using this value for the variance is that, since the CRMLN's primary goal is to evaluate accuracy, we do not want to penalize a system that has very good precision. The effect of using this t-test is that the test system is given some benefit of the doubt. The t-test is performed at various levels for alpha α). A significant bias at α=10% should be interpreted as a warning that the bias is very close to the NCEP criterion. A test system that has significant bias at α=5% is deemed to not meet the NCEP bias criterion and will not pass certification.

The test for within-method outliers is based on the procedure described in NCCLS EP9-A (7). Tests of absolute and relative differences are performed. For the test of absolute differences, the difference between the test method duplicates is calculated for each sample. A test limit is determined which is four times the average difference. Any sample with a difference greater than the test limit is flagged. For the test of relative differences, the difference between duplicates is divided by the test method mean. A test limit is determined which is four times the average relative difference. Any sample with a relative difference greater than the test limit is flagged. Only samples that are flagged by both the absolute and relative tests are within method outliers.

The test for between-method outliers is also based on the procedure described in NCCLS EP9-A (7). Tests of absolute and relative differences between the two methods are performed. For the test of absolute differences, the difference between the test method mean and the reference method mean is calculated. A test limit is determined which is four times the average difference. Any sample with a difference greater than the test limit is flagged. For the test of relative differences, the difference between the test method mean and the reference method mean is divided by the reference method mean. A test limit is determined which is four times the average relative difference. Any sample with a relative difference greater than the test limit is flagged. Only samples that do not pass both tests are between-method outliers.

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CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK

Fee schedule
HDL Cholesterol Certification Protocol for Manufacturers
(using designated comparison method for HDL cholesterol [HDLC DCM])

MANUFACTURERS

A. Fresh Sample Comparison **$4800.00**

1. Duplicate HDLC DCM analysis of 40 patient specimens
2. Shipping charges paid by manufacturer
3. Data analysis for one instrument/method, material, or reagent application
4. One repeat data analysis, if necessary

B. Additional specimens **$120.00/specimen**
(duplicate HDLC DCM analyses)

C. Data analysis for additional applications **$100.00/method**

CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK INFORMATION FORM

The following information form should be completed carefully and accurately. This information will be used to prepare your Certificate of Traceability.
Photocopy this blank form and retain it for future submissions.
Prepare copy of data and retain for laboratory records.
For registered products, please indicate preferred designation: **Registered Trademark®**, *or* **Trademark™**

Laboratory Name: _____

Laboratory Address: _____

_____

Contact Name: _____ Phone: _____

Email address: _____ Fax: _____

Send Bill To: _____ PO# _____

Address (if different from above): _____

_____

_____

Date Specimens Sent: _____ **Date Specimens Received**: _____

Instrument: Manufacturer: _____

Trade Name: _____

Model Number: _____

Calibrator: Manufacturer: _____

Trade Name _____

Lot Number(s): _____

_____

_____

Reagent: Manufacturer: _____

Trade Name: _____

Lot Number(s): _____

_____

_____

Calibrator Set Point(s): _____

_____

_____

Matrix/Sample Type: _____

Anticoagulant (if applicable): _____

Concentration: _____

Separation Method: _____

Precipitation Method: _____

Manufacturer: _____

Trade Name: _____

Lot Number: _____

Homogeneous Method: _____

Principle: _____

CRMLN laboratory complete this section and send the form to Mahnaz Dasti at CDC. Fax: (770) 488-4192. Email: mdasti@cdc.gov

CRMLN laboratory name: _____

Date of Data Analysis: _____ Check One: Passed [ ] Failed [ ]

Director's Signature: _____

CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK

HDL CHOLESTEROL

FRESH SAMPLE COMPARISON RESULTS FORM

Please photocopy this blank form and retain it for future submissions

RUN #1 – Date: _____

ID Number	Result #1	Result #2

RUN #2 – Date: _____

ID Number	Result #1	Result #2

RUN #3 – Date: _____

ID Number	Result #1	Result #2

RUN #4 – Date: _____

ID Number	Result #1	Result #2

CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK

HDL CHOLESTEROL

FRESH SAMPLE COMPARISON RESULTS FORM

Please photocopy this blank form and retain it for future submissions

RUN #5 – Date: _____ RUN #6 – Date: _____

ID Number	Result #1	Result #2	ID Number	Result #1	Result #2

QUESTIONS ABOUT THIS PROTOCOL SHOULD BE DIRECTED TO THE CRMLN LABORATORY

CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK
HDL CHOLESTEROL SPECIMEN DISTRIBUTION FORM

The following chart is supplied to assist you (or the off-site laboratory which supplies you with sera) in selecting specimens that will adequately cover the concentration ranges recommended by this protocol. *[This form is provided as an aid - it is not necessary to return it to the CRMLN laboratory.]*

Please photocopy this blank form and retain it for future comparisons

SPECIMEN DISTRIBUTION FOR HDL CHOLESTEROL METHOD EVALUATION

Conc. (mg/dL)
(Minimum number needed)

	20 – 29 (5)		30 – 39 (5)		40 – 49 (5)		50 – 59 (5)		60 – 69 (5)
1	_____	1	_____	1	_____	1	_____	1	_____
2	_____	2	_____	2	_____	2	_____	2	_____
3	_____	3	_____	3	_____	3	_____	3	_____
4	_____	4	_____	4	_____	4	_____	4	_____
5	_____	5	_____	5	_____	5	_____	5	_____
6	_____	6	_____	6	_____	6	_____	6	_____
7	_____	7	_____	7	_____	7	_____	7	_____
8	_____	8	_____	8	_____	8	_____	8	_____
9	_____	9	_____	9	_____	9	_____	9	_____
10	_____	10	_____	10	_____	10	_____	10	_____

CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK

Quality Control Results Form for HDL Cholesterol

Report single analyses of any quality control material with an HDL cholesterol concentration of 30 - 60 mg/dL (recommended). Data must be obtained with the analytical system under evaluation and must include the runs used in the split sample comparison.

Please photocopy this blank form and retain it for future submissions

Run Number	Date	Result
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Cholesterol Reference Method Laboratory Network

Protocol Checklist

Yes	No	
☐	☐	Have you collected a minimum of 40 samples?
☐	☐	Were the sample concentrations distributed according to the guidelines found in the protocol?
☐	☐	Were all of the fresh samples analyzed in duplicate?
☐	☐	Were the fresh samples distributed among a minimum of five analytical runs?
☐	☐	Were the same instrument, reagent lot, and calibrator lot used in ALL analytical runs of the fresh samples?
☐	☐	Have you submitted data for 20 analytical runs of a quality control material?
☐	☐	Do the 20 analytical runs of the quality control material include the analytical runs of the fresh samples?
☐	☐	Have you completed the Information Form provided in the protocol?
☐	☐	Have you completed the Fresh Sample Comparison Results Form provided in the protocol?
☐	☐	Have you completed the Quality Control Results Form provided in the protocol?
☐	☐	Does your CV for the QC data meet the NCEP goal of < 4%?
☐	☐	Have you provided sufficient volume of serum for the CRMLN laboratory, as described in the protocol?
☐	☐	Have you notified the CRMLN laboratory of your plans to ship samples?

Have you checked "Yes" for each item?
If not, please make sure that you have met all of these requirements before sending samples and data to the CRMLN laboratory. We are not able to analyze samples or data that do not meet these requirements.

CHOLESTEROL REFERENCE METHOD LABORATORY NETWORK

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Methods
DCM: designated comparison method
RM: ultracentrifuge reference method

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